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Preliminary Report

Syncytial Formation Induced by Herpes Simplex Virus *in vitro*

It has been reported that herpes simplex virus induces formation of giant cells *in vitro* as well as *in vivo*.[1,2] Recent studies using monolayer cultures have shown that this pathological change has a close relation with the genetic characters of virus particles.[3-5] This report deals with two problems regarding syncytial formation caused by herpes simplex virus: first, with the effect of the viral dose on syncytial formation and second, with the variation in herpes simplex virus.

a) Effect of the viral dose on syncytial formation

In an earlier paper we reported the effect of the viral dose on the degree of giant cell formation in L cell monolayers.[6] It was found that high virus multiplicities per cell induced earlier and more extensive formation of giant ceells and that a higher concentration of viral material is required to form giant cells than to infect all the L cells in the first cycle of virus growth. However Roizman's recent report on polykaryocytes induced by herpes simplex virus has shown that polykaryocytes are scarce and contain few nuclei in cell cultures exposed to a sufficient dose of virus to infect all or most cells. This finding could not be explained as due to cytotoxic effects of such high virus doses.[7] These results differ from ours.

To clarify this point the following experiment was performed. The supernatant fluid of infected FL cells was used as the viral source. FL cell monolayers were prepared in flat square tubes. Each tube contained 6.0×10^5 cells. Titration of the adsorbed virus was performed by the microplaque method. The original virus sample was diluted with L-E solution by the 10 fold dilution method. Aliquots of 1.5 ml of each diluted viral sample were inoculated into two square tubes and incubated at 37°C for 1 hour. Then the cultures were washed five times with Hanks' BSS and maintenance medium was introduced. The numbers of plaques were counted 48 hours after inoculation. The adsorbed virus titer of the original sample was 7.5×10^7 PFU per 1.5 ml.

To study the correlation between the formation of giant cells and the viral dose of the inoculum, the original virus sample was diluted serially two fold up to $1:2^5$. Aliquots of 1.5 ml of each virus dilution were introduced into two square tubes. The cultures were incubated at 37°C for one hour. Then the inocula were discarded and the cultures were washed five times with Hanks' BSS. Finally maintenance medium (L-E solution containing 5 per cent bovine serum) was introduced. Twenty-four hours later all the cultures were taken out of the incubator and their cytopathic changes studied microscopically. All the cultures showed syncytia, which spread all over the walls of the flat square tubes, but no tendency for rounded mononucleate cells to become more predominate in cultures inoculated with higher virus titer was found. It was found from the titer of original

virus sample that all cells had been infected with a multiplicity of more than 1, even in cultures inoculated with the highest virus dilution, (*i.e.*, $1:2^5$). Under our experimental condition, a high virus input did not cause minimal clumping of infected cells but induced a large syncytium in the sheet. This was consistently found in other experiments also, such as growth curve experiments and serial passage experiments using +GC virus.

The above data may be explained as due to cytotoxic effects of virus concentrates, in contrast to Roizman's data. It is interesting to see from the recent data of Okada that a large dose of HVJ partially inactivated with immune serum causes more severe fusion phenomena in Ehrlich ascites tumor cells than non-inactivated virus.[8] This suggests the possibility that giant cells may be formed by a large dose of partially inactivated virus by "microepidemiological" infection on cell monolayers cultivated in medium containing antibody.

b) Variation of herpes simplex virus

Variants of herpes simplex virus, which were differentiated by the cytopathic changes induced by them *in vitro*, have been reported by some investigators. These are summarized in Table 1. Such phenomena have also been reported with pseudorabies virus and herpes B virus and seem to be generally true of virus of the herpes group.[9,10]

Table 1. Variants of Herpes Simplex Virus Differentiated with Their Cytopathic Effects on Cells

Authors	Date of publication	Type of variant
McNair Scott *et al.*[3]	1959	proliferative ..... P giant cells ..... GC
Hoggan *et al.*[4]	1959	macroplaque..... MP microplaque ..... mP
Nii *et al.*[5]	1961	giant cell producing ..... +GC cell rounding ..... −GC
Nii[12]	1961	giant cell producing .....+GC cell rounding .....−GCr causing fusiform development ...−GCf
Kohlhage *et al.*[13]	in press	?

It is conceivable that each type of variant can change to another type by mutation or by some other mechanism. However, the cause of the appearance of such variants of herpes simplex virus has not been clarified. In our earlier work, we could not decide whether the +GC variant was derived from viral material isolated from a patient or whether it appeared by mutation of −GCr virus during *in vitro* passages of the original virus population. However it was found that +GC type virus had a selective advantage over the parent virus population in serial passages using FL cells.[5] After isolation of a −GCr virus clone in September

1960, the virus was serially passaged on FL cells. In 1961 virus cloning was performed twice with passages between the clonings. These procedures are shown in Table 2. For the past two years both —GCr and +GC viruses have been transfered serially, and have shown these type, but not other types of cytopathic effects on FL cells. Hitherto sixty passages of —GCr virus have been made.

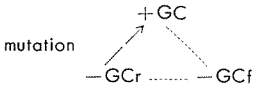
Table 2. Isolation and Reisolation of +GC Variants

Aug. '58	Isolation of the Miyama strain from the vesicular fluid of a patient		
	↓		
	Passages of parent virus population (FL_2, $HeLa_5$, FL_{40})*		
	↓	↘	
Sept. '60	—GCr	+GC I	isolation of +GC and —GCr variants
	↓ ... passages		
July. '61	Viral cloning		
	↓ ... passages		
Nov. '61	Viral cloning		
	↓ ... passages		
Sept. '62	—GCr ——→	+GC II	isolation of +GC variant
	↓		

* FL_2, $HeLa_5$, FL_{40} ... signify virus serially passaged 2 times on FL cells. 5 times on Hela cells and 40 times on FL cells.

Recently, together with the cytopathic effects of the —GCr type, syncytial formation has often been detected in monolayers in test tubes which were inoculated with —GCr viral samples prepared by the limiting dilution method. However no other type of cytopathic effect other than the +GC and —GCr types has yet been found. These data suggest that the +GC variant originated from the —GCr virus population by mutation and increased in number by selection, as described above. (Fig. 1)

Fig. 1. Interrelation between the Three Substrains of the Miyama Strain of Herpes Simplex Virus



The fact that FL cells promote the selection of giant-cell variants of herpes simplex virus was also noticed by Hoggan.[11] Therefore the possibility of finding back mutation of +GC to —GCr seems unlikely on FL monolayers.

At present, two kinds of +GC variants have been obtained. The one was isolated previously and the other has recently been isolated. The former was designated as +GCI, and the latter as +GCII. Morphologically, cytopathic effects induced by both were the same. Comparative studies on the growth kinetics of the two are now in progress.

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